Lamprey Ammocoete Sediment Bioassay Recommendation
Portland Harbor Natural Resource Trustee Council Fish Committee
November 9, 2007

Introduction

Lamprey play an important role in the ecosystem of the lower Willamette River and are an important resource for the tribes. Lamprey provide a vehicle for recruitment of marine nutrients to the streams in the system, as well as a buffer for avian (Merrell 1959), marine mammal (Jameson and Kenyon 1977; Roffe and Mate 1984), and picivorous (Beamish 1980, Poe et al. 1991) predation. Lamprey are used by native Americans of the Pacific Northwest and annual collections continue at Willamette Falls. Populations of lamprey have declined in recent history; numbers of Pacific lamprey at fishway counting stations in the Columbia River Basin are currently about 3 to 5% of what they were 50 to 60 years ago, indicating a dramatic decline in lamprey abundance. A priority for natural resource agencies and tribes is to minimize the risk of contaminant exposure to lamprey in the Portland Harbor and conduct restoration activities that are beneficial to lamprey.

Under the remedial investigation and risk assessment, lamprey are identified as receptors to be protected at the individual level. Life history information for lamprey ammocoetes within the Portland Harbor is sparse, and very little toxicity information is available to be able to assess this species at the individual level using no-effect toxicity thresholds. Because there are so limited data, EPA sought additional information to support the risk assessment for lamprey, including two major components: a lab-based exposure of ammocoetes to selected substances to attempt to estimate the relative sensitivity of ammocoetes to those substances compared to other fish for which protective TRVs have been determined, and a survey of the extent of contamination in the tissues of ammocoetes in the study area. In this memo, we propose an additional, straightforward, sediment toxicity testing study using ammocoetes to remove many of the uncertainties that will not be addressed by the previous studies.

Basing the risks on comparisons to the responses of other fish in short-term, water-only exposures leaves substantial uncertainties because of the unique physiology and life history of lamprey. Lamprey have a long evolutionary history with a direct descendancy from some of the most ancient vertebrates (Youson and Sower 2001). Although lamprey are native to the Pacific Northwest and are sympatric with salmonids, their different physiology and habits could make them more susceptible to contaminant exposure compared to teleost fish. Lampreys have unique CO_2 exchange proteins in their blood cells (Cameron 1999) and digestive enzymes (Venkatachalam et al. 2004) not found in salmonids. Lamprey ammocoetes also regulate thyroid hormones differently than other fishes during critical life stages such as during metamorphosis (Youson and Sower 2001). Hahn et al. (1998) reported that lamprey do not exhibit measurable ethoxyresorufin *O*-deethylase activity in the presence of 3,3´,4,4´-tetrachlorobiphenyl (a potent aryl hrdrocarbon receptor agonist in other teleost and cartilaginous fishes). Habitat requirements for lamprey ammocoetes also are very different compared to other fish. Ammocoetes lack swim bladders and are found within the sediment in areas with both low and high dissolved oxygen (DO), where they filter feed detritus and are relatively sedentary for periods of time. Of perhaps more relevance are studies that show lampricides are selectively toxic to lamprey because

lamprey lack the systems found in teleosts to detoxify the lampricide (USFWS 2001), and that lamprey ammocoetes and adults accumulate mercury differently than teleosts, with ammocoetes having higher than expected concentrations (Drevnick at al. 2006).

Lamprey are also unique compared to other fish in the Portland Harbor because they undergo a true metamorphosis from ammocoete to the juvenile stage, which occurs while residing in the harbor. This stage does not occur for any other fish in the harbor. Lamprey ammocoetes may reside in Portland Harbor sediment for years, sufficient time to feed and grow. During this early life stage, lipogenesis exceeds lipolysis and lipid is accumulated in storage sites (Sheridan and Kao 1998). This first stage represents a potentially sensitive period where lamprey bioaccumulate exogenous lipohilic compounds from their surroundings. Composite samples of ammocoetes collected in 2006 from Portland Harbor exhibited concentrations of polychlorinated biphenyls (PCBs) that were 8.8 times higher than concentrations in upstream samples, and DDT was 1.5 times higher than the upstream concentrations. These residue data provide evidence that ammocoetes are bioaccumulating lipohilic compounds to a greater extent while in the harbor compared to lampreys that have not yet reached the harbor and are still moving downstream, and exposure in the harbor occurs during the first stage of metamorphosis. During the second phase of metamorphosis, lipid lipolysis is greater than lipogenesis and lipids are depleted from storage sites (Sheridan and Kao 1998). During this stage (which also can occur while lamprey reside in the harbor), lipophilic contaminants are metabolized during lipolysis and can expose the developing lamprey during a sensitive period to compounds that are capable of disrupting hormones and impeding growth. For example, Leney (2006) reported the chemical activity of PCBs in green frog tadpoles was increased during metamorphosis, thereby exposing the tadpoles to a contaminant during a sensitive development stage. In sea lamprey, lipogenesis is promoted by insulin and by thyroid hormones, whereas lipolysis is promoted by hormones including prolactin, growth hormone, andrednocorticotropic hormone, corticosteroids, somatostatins, and thyroid hormones (Sheridan and Kao 1998, Youson 1997). Normal development in lamprey ammocoetes "requires a coordinated regulation of development-associated changes in lipid metabolism that results from hormone interactions and other internal and environmental clues" (Sheridan and Kao 1998). Because some lipohilic compounds present in the harbor such as polybrominated diphenyl ethers, DDT compounds, PCBs, and some metals can disrupt thyroid and other reproductive and growth hormones, lamprey ammocoetes are at risk of experiencing growth and development impacts during metamorphosis from compounds in Portland Harbor that are bioaccumulated into lipids and then metabolized during second stage metamorphosis. The exposure pathway and potential sensitivity are unique to lampreys due to their process of metamorphosis while residing in sediment within the harbor, and current threshold reference values (TRVs), based on short-term, water-only tests, used to evaluate the sensitivities of teleosts would not represent the exposure pathway or long-term responses unique to ammocoetes.

Sampling during the Round 3A field effort determined that lamprey are present in the Portland Harbor at all early life stages and accumulate at least some organic contaminants such as DDT and its transformation products, PCBs, and polynucleararomatic hydrocarbons (PAHs) to a much higher concentrations than ammocoetes sampled upstream. However, there are many data gaps remaining since the Round 3A field collection of ammocoetes. Lamprey ammocoete sampling during the Round 3A field effort was conducted to determine if contaminant concentrations in field-collected lamprey exceeded upstream concentrations or tissue-based TRVs and therefore

pose risk to lamprey themselves, and to characterize tissue concentrations in areas with elevated sediment concentrations, in quiescent and high-flow areas, or in areas of special interest to EPA. Because too few lamprey were collected, only the objective of comparing concentrations to upstream values could be addressed. Results showed accumulation of some organic compounds in lamprey in the harbor, but only the concentrations of PCBs in ammocoetes exceeded a conservative effects threshold established by Meador et al. (2003) for protection of juvenile salmon. Whole-body TRVs for fish for other substances were not exceeded, but due to potential sensitivities and exposure pathways unique to lamprey as indicated above, the established fish TRVs may not be appropriate to use for comparison to lamprey. Also, lamprey from broad areas of the harbor were composited to achieve adequate sample mass for analytical tests, but lamprey were not collected from locations with higher concentrations of the tested chemicals in the sediments (see attached figures), so only limited inference can be gained on what tissue concentrations might be observed in ammocoetes exposed throughout the study area. Specifically, it is unknown if ammocoetes exposed to sediments from sites with elevated sediment contamination would have tissue concentrations exceeding established fish-based TRV values. It also remains unknown if the paucity of lamprey found in the harbor was due to inefficient sampling methods, lack of suitable habitat, or because the presence of contaminants prevented colonization of lamprey entering the area from upstream.

For the reasons noted above, using data from other fish species as surrogates for lamprey may not adequately represent risk to lamprey in the Portland Harbor—both their responses and exposure pathways are different from pelagic or demersal fish. Because the ammocoetes complete their early stages within the sediment they are more accurately considered to be benthic organisms. However, the other benthic organisms addressed in the risk assessment, including those used in direct toxicity tests in the Portland Harbor sediments, may not be representative of a fish species. Further, the risks to those benthic organisms will be evaluated differently than for fish species. Finally, other species, such as clams, from the study area may bioaccumulate toxic substances in a manner similar to lamprey, but ammocoetes accumulation compared to other species has received limited study. At a minimum, ammocoetes have greater lipid content and likely have different sensitivities compared to these other benthic organisms.

Information collected directly on lamprey ammocoete/site-sediment interactions in sediment exposures of ammocoetes to site sediments, as proposed herein and described below, will reduce the uncertainties associated with the current surrogate-species approach and directly address risks to individuals of the species to the specific contamination exposures present in Portland Harbor. It is expected that this proposed study employing toxicity testing with ammocoetes and site sediments will address many of these remaining data gaps and provide information that will allow comparison of ammocoete sensitivity to results from toxicity tests with benthic invertebrates, reduce the uncertainties in the Phase 1 and 2 water-only toxicity tests, provide characterization of lamprey sensitivities or degree of tolerance in areas with elevated contaminants in sediment, bridge data gaps in assessing appropriate TRVs and provide a corrective value (if needed) for TRVs established for other fish species or benthic invertebrates, and establish biota-sediment accumulation factors for some organic contaminants. In the usual EPA risk assessment process, performing bioassays with site sediments is one of the more common measurement endpoints for the assessment of sediment-associated species. The proposed bioassays would provide a direct measure of the risks to an assessment endpoint of the

survival and normal growth of lamprey. Similarly, as noted in a previous memo, the sediment bioassays could satisfy many of the DQOs that have been previously identified and agreed to for lamprey.

Approach

The testing method suggested for the sediment bioassays with the ammocoetes is based on minor modifications to standard 28-day *Hyalella* sediment bioassay, already approved for the testing of site sediments (Integral et al. 2004), using ammocoetes collected from the "clean" locations being used to provide fish for the Phase 1 and 2 water-only toxicity tests. The successful collection and holding of ammocoetes for the Phase 1 water-only range-finding tests demonstrates that there are no husbandry issues to limit the testing. Bioaccumulation testing would be performed by collecting the ammocoetes surviving at the end of the test exposures and measuring the concentrations of selected bioaccumulative substances in their tissue. Modifications and specific issues would include:

- Ensuring an adequate depth of sediment in the test chambers: 7 to 8 inches of burrowing sediment should be used.
- Selecting the ammocoete size/age: as was agreed with the water-only tests, ammocoetes in the size range of 2.5 to 4.0 cm should be used.
- Source of ammocoetes: Ammocoetes would be collected from similar locations as those used for the Phase 1 and 2 water-only tests.
- Temperature: The tests should be run at 17±1º C and the ammocoetes should be collected at stream temperatures between 16º C and 20 ºC.
- Quantity (numbers/biomass): For testing toxicity only, 10 ammocoetes per replicate test chamber should be used. For bioaccumulation chemical testing, if each replicate were tested separately, 60 of the small ammocoetes would be required per replicate (assuming an average ammocoete wet weight of 0.5 g and 30 g required for the chemical analyses).
- Length of exposure: The test should be designed to run for 28 days.
- Control and reference test sediments: Control test sediment could be either a tested commercial sand or sediment from one of the ammocoete collection sites. No reference sediment need be used.
- Endpoints: The test endpoints would be survival and growth, as for the other sediment bioassays. Incidental behavioral observations, e.g., initial ammocoetes burial behavior and ammocoete that have left the sediments, should be made daily.
- Bioaccumulation: At the end of the test, surviving ammocoetes would be collected, washed of external sediment, placed in pre-cleaned sample jars, frozen, and shipped to an analytical lab for analyses of bioaccumulative substances: selected PCB congeners, selected polychlorinated dioxins and furans, selected organochlorine pesticides, selected PAHs, mercury and other selected metals, tributyl tin, lipids, and percent moisture.

Locations/Scale/Numbers of Tests/Samples

The site sediments for the testing should represent the range of contaminant types and concentrations observed in the study area, provide adequate spatial coverage of the sediments throughout the study area, and have been or will be used for bioassay testing with the other benthic organisms. Within those criteria, sediments should be collected that have appropriate

grain size, organic content, and composition. Standard procedures can be applied to reduce the concentrations of ammonia or sulfide in the tests, if necessary.

Because of the extent of supporting information from the water-only bioassays, the other sediment toxicity tests, and the site characterizations, ammocoete testing at 50 locations is considered appropriate. This number of sites was selected to ensure that the samples covered an adequate range of the selection variables noted above.

These 50 sites would be selected from throughout the study area in locations that have been chemically and physically characterized, that are considered "representative" of the contamination. In addition, to maximize the potential data usability, sites should be selected that have been or will be tested with the other sediment bioassays, and include locations with a range of responses by the other organisms.

The specific sediment samples collect for the ammocoete testing would be chemically and physically characterized as has been done with other test samples.

Results

The results of the toxicity testing would be analyzed statistically to elucidate the dependence of the ammocoetes responses on the substances present in the sediments and their concentrations, any dependence on the sediment physical characteristics, and the sensitivity of the ammocoetes compared to the organisms used in other sediment bioassays. In addition, the responses would be mapped by location. It is anticipated that the most straightforward use of the data will be to determine the relative sensitivity of the ammocoetes to the sediments in comparison to the benthic invertebrates used in similar, but more extensive, bioassays. Clean-up concentrations developed from the latter data could then be convincingly applied to ensuring that those clean-up goals will be protective of lamprey.

Depending on the quality of the results, it may be possible to develop site-specific sediment TRVs for the ammocoetes, and therefore provide information to characterize toxicity on a site-specific basis throughout much of the study area including areas of particular interest to EPA risk assessors. Similarly, the comparisons to the results of the sediment bioassays with the other organisms may be sufficiently consistent to use those other data as well to more accurately estimate the risks to ammocoetes in many other areas.

The bioaccumulation results would be tested statistically to determine whether (and for which substances) bioaccumulation can be shown to depend directly on the concentrations of the substances in the sediments with the aim of developing BSAFs for the site.

Schedule

The only substantial constraint on performing the sediment bioassays with ammocoetes is the seasonal times when ammocoetes can be collected to support to lab exposures. There may also be permit restrictions that limit the availability of ammocoetes, particularly for this year. Some

cost savings may be possible by performing the testing coincident with other projects that require the collection of sediments.

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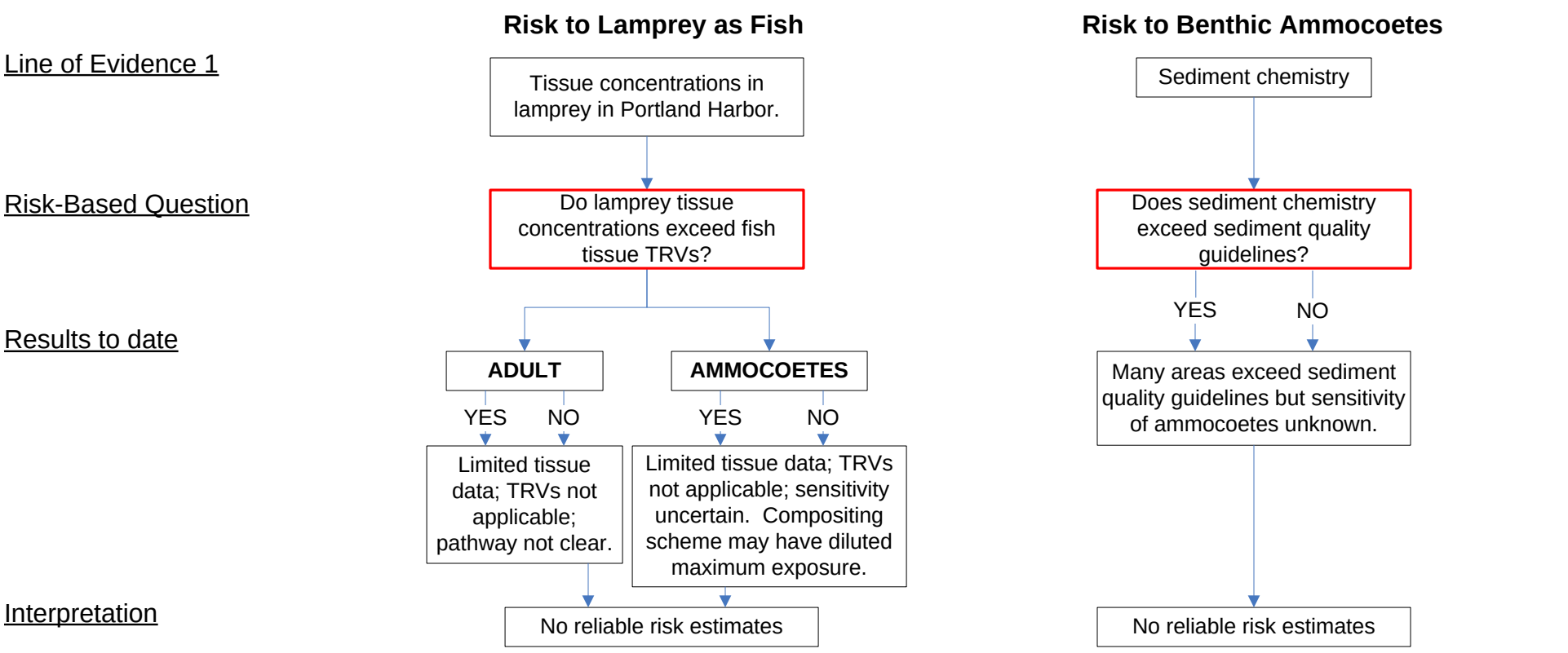
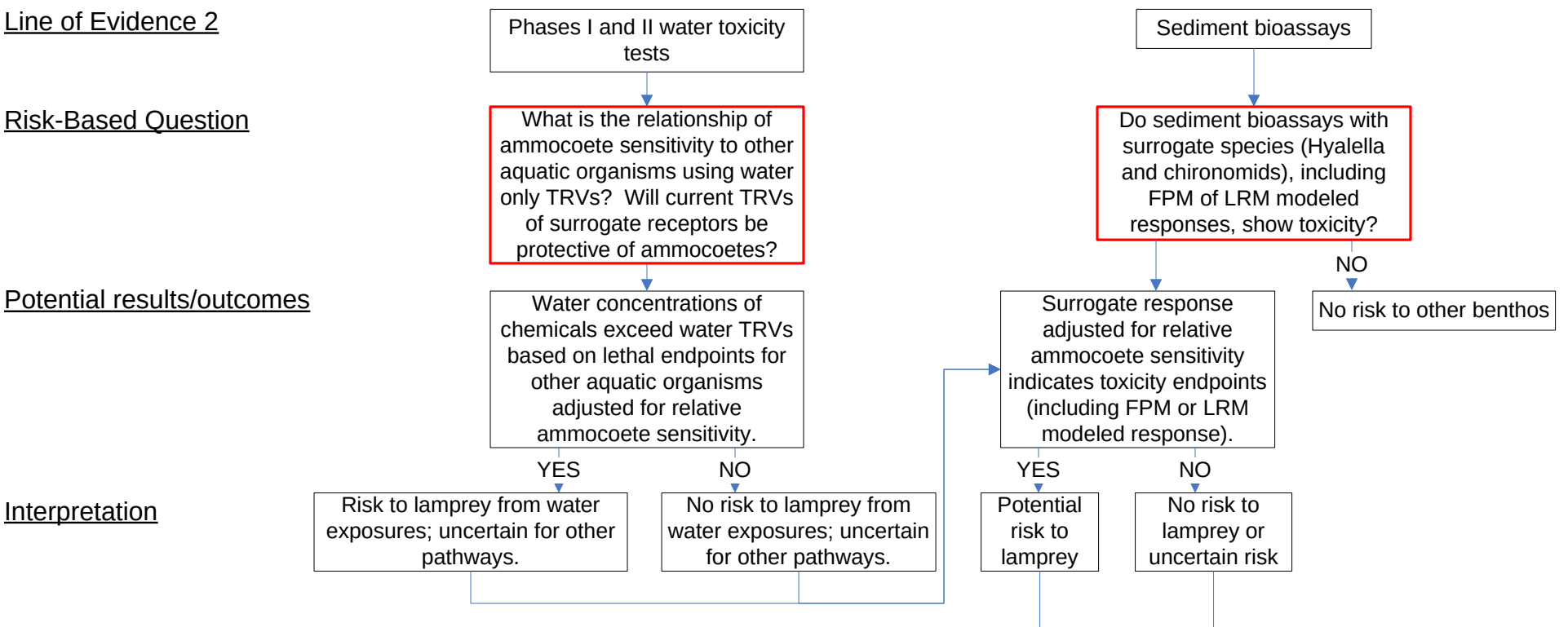
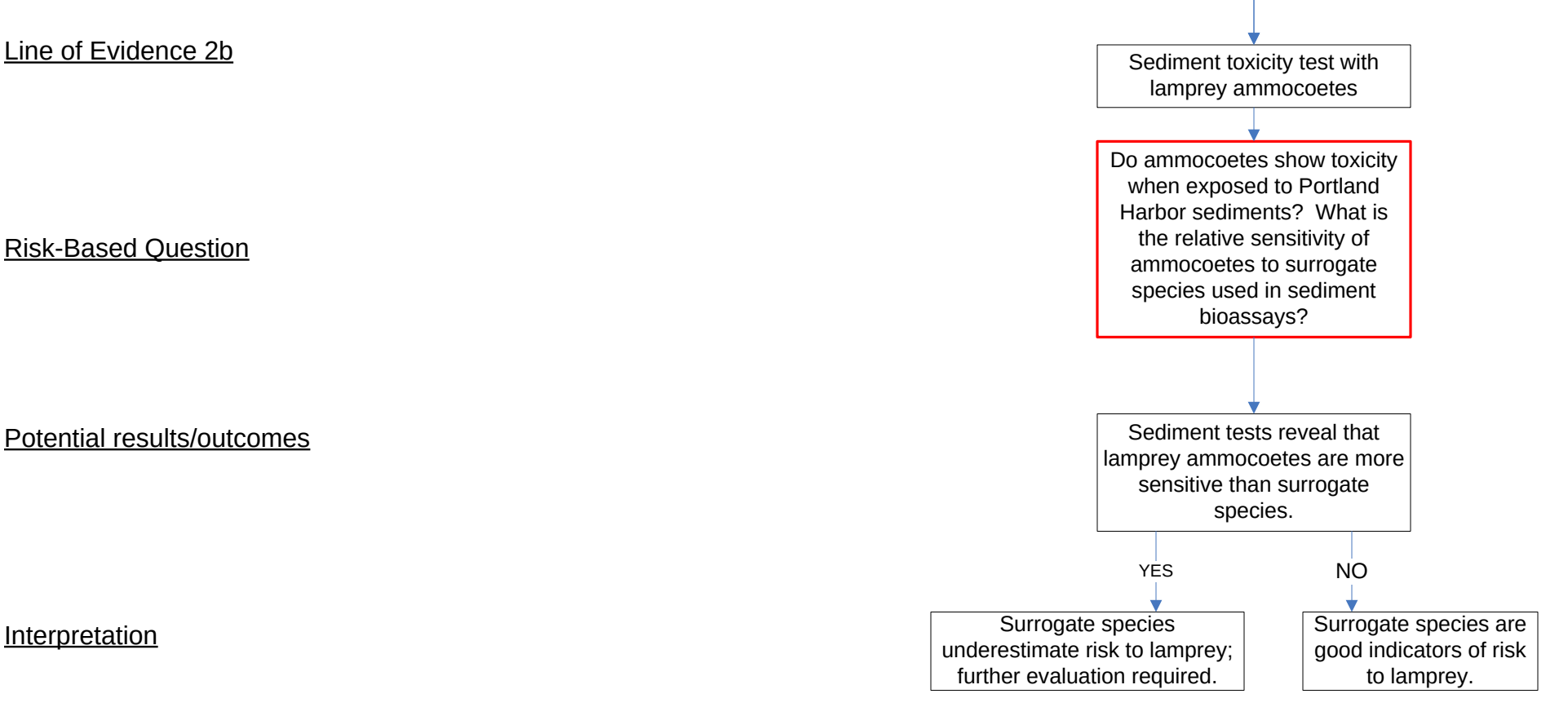
Lamprey Risk Decision Tree



Conclusion from Line of Evidence 1: Additional lines of evidence needed to reduce uncertainty



Conclusion from Line of Evidence 2: Potential risk to lamprey, no risk to lamprey, or uncertain risk



Conclusion from Line of Evidence 2b: Definitive estimate of risk to ammocoetes to meet DQO's for remedial investigation

* Sediment test protocols and feasibility would be established in preliminary pilot test.

Draft Protocol Outline for Preliminary Ammocoete Sediment Bioassays

The primary purpose of the trial run is to determine the feasibility of conducting sediment toxicity tests with ammocoetes over a 28 day period and evaluate any problems that arise during the tests. Secondarily, it is to determine if observation of toxic endpoints (reduction in growth or survival) from exposure to sediments considered to be toxic is possible. The purpose of this test is explicitly *not* to determine the sensitivity of ammocoetes relative to other receptors; rather, it is to ensure that measurement endpoints can be observed in a manner consistent with and comparable to other sediment bioassays types/results.

Select 3 sediments from those collected for other bioassays

- Use preliminary sediment chemistry data to select a range of concentrations and texture (ensuring at least one sediment sample is from an area that has shown toxicity in other tests)
- Alternatively, use previous results to select the sediments

Prepare test chambers (beakers)

- Add 4 (to 6) inches of test sediment to each of 3 replicates
- Add overlying hardness-adjusted lab water (as used for water-only bioassays)
- Run tests at 17ºC, 25 mg/l hardness in overlying water, and 16/8 light/dark cycle
- Use the commercial sand used for acclimation and maintenance of the ammocoetes for the control replicates

Add 5 ammocoetes, remaining from previous testing that have been acclimated and maintained in commercial sand, to each test and two sets of control replicates

- Try to randomize ammocoete sizes among the test chambers
- Weigh the ammocoetes prior to placement

Run test for 28 days

- Maintain overlying water quality with aeration and static renewal
- Monitor water temperature, dissolved oxygen, and hardness
- Feed the ammocoetes weekly with yeast
- Monitor the ammocoete activity daily
- Determine whether normal behavior allows ammocoete survival to be monitored daily without disturbing the ammocoetes
- Determine if ammocoetes can be counted reliably at periodic intervals based on filtering behavior
- Using one set of the control replicates, test procedures for safely removing ammocoetes at days 10 and 20 to verify survival and to weigh the ammocoetes
- Identify “abnormal” behaviors, e.g., leaving the sediment

At end of tests

- Count living ammocoetes
- Weigh living ammocoetes
- Option: combine the ammocoetes from all 5 test replicates and have tissues analyzed for selected substances